FURTHER OBSERVATIONS ON AXIAL SUSCEPTI-BILITY GRADIENTS IN ALGÆ.

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WITH TWO FIGURES.

INTRODUCTORY.

In a recent paper (Child, '16a) it was shown that axial gradients in susceptibility to cyanides and various other agents are characteristic features of some fourteen species of axiate marine algæ, and the question of the significance of these gradients in relation to polarity and developmental order was considered.

The present paper records observations made during the summer of 1916 at the Marine Biological Laboratory, Woods Hole. It is concerned primarily with the demonstration of what may be called the normal gradients in the species examined, *i. e.*, the gradients characteristic of the plant in good physiological condition under good or average rather than extreme, environmental conditions, but some of the alterations resulting from altered environment are briefly described. In addition to the fourteen species of the earlier paper, eleven more species have been examined, at least in part, with definite results in every case.

The method used is essentially the same that was employed to demonstrate the axial gradients in various other animal and plant species (Child, '13b, '14, '15a, '15c, Chap. III., '16a, '16b). It consists in determining the susceptibility to, *i. e.*, the survival time in, a certain concentration of an agent which kills within a few hours, but not immediately. The differences in susceptibility as determined by the differences in survival-time along an axis or in different organs are in general an indication of the differences in physiological condition. The relations between susceptibility to inhibiting agents and physiological, metabolic or protoplasmic condition have been discussed elsewhere (Child, '13a, '15b, Chap. III., '16a) and require no further consideration at present.

The time of death of the plant cells is approximately deter-

mined by the visible changes in aggregate condition of the proto-
plasm, and in many of the red and brown algæ by the diffusion
of the pigment out of the chromatophores and out of the cell.
The protoplasmic changes are in many cases much more readily
seen if the plant has been previously stained with neutral red.
The general character of the death changes has been described
(Child, '16a) and some special observations are recorded below.

In the work of 1915 the chief agents used to measure suscepti-
bility were KCN, ethyl alcohol and the so-called vital dye, neutral
red. In 1916 various other reagents in addition to these three
were used, including ethyl ether, HCl, $CuSO_4$ and $HgCl_2$.

The Susceptibility Gradients in the Thalli.

The chief result of this further study is the same as that of the
earlier, viz., that in the definitely axiate forms or parts examined
a gradient in susceptibility exists along the axis, the apical
region being primarily most, the basal least susceptible to toxic
agents in high concentration. The regularity of this gradient
is most marked in plants in good physiological condition and in
the younger axes or the younger portions of axes. In some
forms the original gradient may persist throughout the length of
the axis, at least during the vegetative period, while in others
it may undergo modification in the later stages or in the older
regions of the body. In general it is also true that the more
definitely axiate and orderly the growth form of the plant, the
more definite and regular the susceptibility relations between
different parts.

Since different species behave somewhat differently and require
different modifications of method the data for each form examined
are briefly given. The genera include, among the Chlorophyceæ,
only *Bryopsis* and *Cladophora*, among the Phaeophyceæ, *Fucus*,
and among the Rhodophyceæ, *Chondrus*, *Cystoclonium*, *Agard-
hiella*, *Lomentaria*, *Griffithsia*, *Callithamnion*.

Bryopsis plumosa.

The demonstration of an axial gradient in this form seemed
to me of particular interest since the whole plant body consisting
of creeping rhizome-like axes from which arise vertical axes with

a highly orderly pinnate arrangement of lateral branches is a single cell. Unfortunately, owing to scarcity of material, it was possible to examine only a few of the vertical axes with their branches and these had been in standing water in the laboratory for twenty-four hours before they were available. They were first stained in neutral red and then placed in KCN $m/50$ in Syracuse dishes covered with a thin glass plate and the course of death observed under the microscope.

In those axes which were still in good condition death began in general at the apical end of each main axis and branch and progressed basipetally and in each system of main axis and branches as a whole a similar gradient appeared, the branches nearest the apical end being most susceptible and death progressing basipetally from branch to branch. Moreover, at least the younger branches were more susceptible than the level of the axes from which they arose.

It would, I think, be difficult to find a more beautiful example of intracellular axial gradients in susceptibility than in *Bryopsis*. As in other forms (Child, '16a) the first indication of approaching death is a deepening of the neutral red tint in the cell as if the protoplasm were becoming more acid. This change in color occurs first apically and progresses basipetally and is followed in a few moments by the disintegrative changes in the protoplast. The progress of the coagulation and aggregation of the protoplasm into masses which are at first almost black in consequence of the high concentration of the neutral red in them, but which lose the stain soon after coagulation, can be followed under the microscope from one level to another as a visible wave of change.

As stated above, the course of death is in general basipetal, but in the few axes examined there was none which did not show some irregularities. In young growing axes the irregularities are much less frequent than in old, where most or all of the branches have completed their growth, and a larger or smaller number of the more basal branches may be in part or entirely dead when the plant is collected. Similarly, the more apical younger portions of an axis with its primary branches usually show fewer irregularities than the older more basal regions. Injuries of course alter the gradient for a greater or less distance

from the part concerned. Where a main axis or a branch has been bent sufficiently to crush or injure the protoplasm the susceptibility is very high unless the protoplasm is already killed, and death usually proceeds in both directions from such a point of injury, but its progress basipetally is usually the more rapid. In general the older parts of the thallus are likely to have received a greater number of such injuries than the younger and the more frequent irregularities in the gradients may be due in part to this, but there is no doubt that with the slowing down of the activity of the apical region of an axis, the gradient undergoes a leveling down and slight local differences in activity in different regions of the cell may determine irregularities in the course of death.

In a plant so delicate as *Bryopsis* it would probably be very difficult to obtain an axis with its branches which would show a perfect basipetal death gradient in all parts. Not only the greatest care in collecting and handling but also absence of injury and a fairly uniform environment for at least a considerable period before collection would be necessary conditions. The point of interest is not the appearance of local or regional irregularities, which are to be expected, but the general regularity.

There can be no doubt that the uninjured axis of *Bryopsis*, in good physiological condition, whether it is a lateral branch or a main axis, shows a basipetal susceptibility gradient, *i. e.*, a gradient in which the progress of death is basipetal and that each system of axis and primary branches as a whole shows a similar gradient.

In my material, which had remained in the laboratory for twenty-four hours before I obtained it, death was already beginning in some of the axes, undoubtedly in consequence of laboratory conditions, as no special care had been taken to keep the plant in good condition In all such cases the dead parts were readily distinguishable from the living by their failure to stain with neutral red, and it was observed that such death began apically and progressed basipetally in each axis and system of axes, *i. e.*, the susceptibility gradient was the same as in KCN. Scarcity of material made it impossible to test susceptibility to other agents and conditions but the observations and experiments on other species leave no doubt that the axial gradients in

susceptibility to KCN are simply a special case of a very general relation between axiate organisms and their environment.

Cladophora sp.

Various specimens collected at various times, first stained with neutral red, then killed in KCN $m/50$, show a basipetal gradient in staining and in death and decoloration.   Apical regions stain most rapidly and most deeply and staining progresses in general basipetally.   Death in KCN also begins apically and progresses basipetally.   Of course exceptions to this general rule appear frequently, particularly in the older parts of the plant, where the gradient has become less distinct, and environmental factors may have affected one cell or another, or a group of cells.   Nevertheless, the general basipetal course of death is apparent even to the naked eye in plants previously stained with neutral red.

As in *Enteromorpha* (Child, '16a) a branch is in general more susceptible than that level of the axis from which it arises. Death progresses to the base of the branch and the cell of the axis from which the branch arises usually dies considerably later.

As its ability to live under infavorable conditions would suggest, *Cladophora* is very insusceptible to KCN.   In KCN $m/50$ death of the apical cells begins only after several hours and the basal regions of the main axes die only after 20–30 hours.   In this respect it contrasts sharply with *Bryopsis* where death in KCN $m/50$ begins in $\frac{1}{4}$–1 hour and the whole plant is dead in 2–4 hours.

When *Cladophora* is killed in a sea-water solution of neutral red alone, death and decoloration are much less rapid than in KCN, requiring several days for completion, but the point of chief interest is that the death gradient is the reverse of that in KCN $m/50$.   Death begins in the basal region, progresses acropetally in each axis and the apical cells are the last to die.   This reversed gradient is like the acclimation gradient (Child, '13a, '13b, '15c, Chap. III.) observed in animals, where the rapidity and degree of acclimation vary directly with the rate of metabolism or physiological condition in different regions when the concentration or intensity of the external agent is not too high. In true acclimation, however, there is more or less approach to

the metabolic rate existing before the action of the external agent and it is not yet certain that such a change occurs in this case. Certain reversals of the gradient in *Griffithsia* described below (p. 430) where true acclimation is out of the question, show that reversal does not necessarily mean acclimation.

In *Enteromorpha* also, where the susceptibility gradient to high concentrations is like that of *Cladophora*, basipetal (Child, '16a) a reversal of the gradient often appears in neutral red, and in various other species more or less reversal has frequently been observed in neutral red. These and other cases of reversal are discussed in a later section (p. 436).

One series of observations made on portions of a single plant of *Cladophora* gave results very different from those recorded above as regards neutral red. Portions of this plant stained with neutral red showed no decoloration even after a week or ten days, although to judge from the contracted and disintegrated appearance of the protoplasm death had undoubtedly occurred. Other portions stained and then killed in alcohol 10 per cent. and 5 per cent. and in ethyl ether 4 per cent. likewise showed no decoloration during three or four days, as long as the preparations were kept, although the altered appearance of the protoplasm even after a few hours gave every indication that death had occurred. In these cases the apical regions for a length of several cells were stained an opaque black, and other portions were deep purple, the color indicating a much higher acidity within the cells than that usually observed. Portions of the same plant in KCN $m/50$ after staining with neutral red showed the usual basipetal decoloration gradient and were completely decolorized after twenty-four hours like other specimens examined.

This case occurred at the end of my stay at Woods Hole so that there was no opportunity for further tests and it is mentioned here only because of the possibility that others, attempting to repeat my experiments, might obtain such results as these. The same behavior as regards neutral red was observed once before in a single test of a fresh water species of *Cladophora*. In neutral red no decoloration occurred even after two weeks, although the plant was undoubtedly dead. Further investiga-

tion is necessary to clear up this apparently anomalous behavior which differs from that of other algæ examined as well as of other specimens of *Cladophora*. It is possible that these peculiar results are due to the neutral red rather than to the *Cladophora*, for with both the fresh-water and the marine form the neutral red used was a different preparation from that which had been used in other cases.

Fucus vesiculosus.

In this species young plants ranging in length from 12–15 mm. to 40 mm. constituted the material. Early in the course of experiments with this form it was found that the change in color and loss of the natural pigment of the plant was a more satisfactory indicator of differences in susceptibility than the decoloration after staining with neutral red and the results described below were obtained by this method.

In the earliest stages of development the plant is more or less club-shaped and circular in cross section, but in consequence of change in behavior of the apical cell the thallus soon assumes a flattened form except in the basal region, and a thickened midrib develops (Fig. 1). The plant is not, properly speaking, bilaterally symmetrical, since there is no differentiation of dorsal and ventral surface, but it is biradial, *i. e.*, there are two distinct axes of radial symmetry, one parallel, the other at right angles to the flattened surface. Since the plant grows primarily from an apical cell situated in the median apical region and since secondary growth in thickness occurs along the midrib, we might expect to find the regions of highest susceptibility apical and median and susceptibility gradients extending laterally and basally from these points, perhaps modified in the more basal regions, at least in later stages by the increased activity of secondary growth. Such a gradient appearly very clearly with various agents.

Sooner or later dichotomous branching begins (Fig. 2), each branch growing from a new apical cell which arises from the original apical cell of the axis undergoing dichotomy. Each branch then may be expected to exhibit the same sort of gradient as the original unbranched thallus and this is actually the case.

The natural color of the thallus is a dirty greenish brown or

yellowish brown. The color gradients have been determined in KCN $m/50$, alcohol 10 per cent., ethyl ether 4 per cent., and HCl $m/100$, and they are essentially similar in all, though the color changes differ somewhat with different agents. In all these agents during the first 3–4 hours there is a distinct loss of
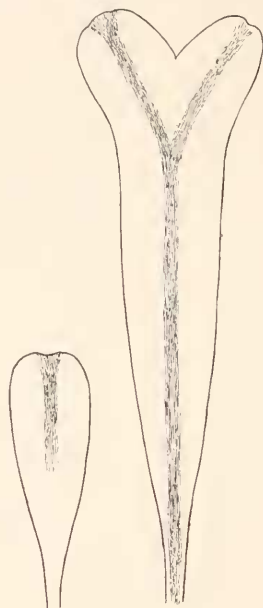
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FIG. 1.   FIG. 2.

color beginning in the median apical region and progressing laterally and basipetally. This change involves the apical 5–8 mm. of the plant and does not progress further, but shades off basally into a deeper color. After this change the apical region for several millimeters is light grayish green (KCN), yellowish green (alcohol), first deep olive green soon becoming yellowish or whitish green ·(ether), or a dirty whitish yellow (HCl). Evidently the pigment in this region diffuses out to a considerable extent and the water is often tinged by it. This pigment loss decreases with increasing distance from the apical end, hence the color gradient from light yellowish or whitish apically to deep color approaching normal basally.

In the course of 10–18 hours in KCN, alcohol or ether the median apical region begins to show a deep brown or reddish brown color, and this color eventually progresses basipetally along the midrib, and a brownish tint spreads laterally over the thallus, the apical regions preceding, but the midrib always remains more deeply brown. In HCl this secondary change is not so clearly marked, the midrib merely becoming more yellowish than the lateral regions of the thallus.

In all cases the color changes begin in the median apical region and progress laterally and toward the base. It is impossible to determine just when death occurs at any particular level of the body in these various agents but it is probably either when the first color changes begin or when the loss of color occurs. The first change in color must mean that the reagent has penetrated the cells at least to some slight extent, and the loss of color must mean that the protoplasm or the pigment or both are so altered that the pigment is no longer held and diffuses out into the water. There can be little doubt that the cells in the regions concerned are dead when this occurs.

The point of chief interest at present, however, is the progress of the changes from the median apical region laterally and basally. This progress is more distinct in the more apical region than elsewhere, and it is in this region that the change from the embryonic to the differentiated condition is most marked. In some cases among the older thalli the cylindrical stem is apparently slightly more susceptible than the basal part of the flattened thallus, perhaps in consequence of the activity of secondary growth in thickness. The reddish brown color is probably a secondary change after death, rather than a death-change properly speaking, but its progress from the median apical region basipetally and laterally is none the less interesting, as indicating still another aspect of the axial gradients.

Callithamnion.

From the physiological point of view it seems best to describe the gradients in Rhodophyceæ with primarily monosiphonous thallus and large cells before taking up species with more complex axiate structure.

The earlier data on *Callithamnion* (Child, '16*a*) are supplemented by observations on two more species, *Callithamnion Baileyi* and a species resembling *C. Baileyi* in sympodial growth-form but with a somewhat different order of branching and strictly monosiphonous throughout, which it was impossible to identify with certainty.

In both these species the primary gradient in each unbranched axis and each cell is basipetal as tested by killing with neutral red alone, with KCN of various concentrations after neutral red and by $HgCl_2$ *m*/50,000. The basipetal gradient in single cells even along the main axes is very distinct and shows few irregularities in plants which are in good vegetative condition, provided they are not killed too rapidly.

As an axis gives rise to branches, however, the primary gradient undergoes certain modifications which are very evidently associated with the growth-form. These modifications concern the susceptibility relations of different branches along an axis. In general the farther from the apical end a branch arises the higher its susceptibility, and the rate of growth of the branches shows the same relation to the axis as the susceptibility, *i. e.*, the more basal branches grow more rapidly than the more apical. Both of these features are associated with the sympodial growth-form of these species and they are the reverse of the relations observed in the monopodial *C. roseum* (Child, '16*a*). A more extended account of these modifications of the susceptibility gradient is postponed to another time.

Griffithsia.

Griffithsia bornetiana, to which my attention was first called by Professor Osterhout, has proved to be one of the most interesting forms thus far examined, first because of the large size of the cells and the conspicuous character of the death changes, and second because the gradient very readily undergoes alterations, both in nature and under experimental conditions.

To the naked eye the color of the plant is usually rather more reddish or less brown than that of most related forms. The cells of the monosiphonous axis are readily visible to the naked eye, the longer more basal cells being often several millimeters in

length. Microscopically by transmitted light the color may be described as a brownish pink. The cells are translucent and the chromatophores and numerous nuclei are readily seen. Before death the cell surface undergoes certain changes in appearance resulting from the aggregation of minute granules or semi-fluid particles, this change differing somewhat in degree in different cells, even of the same plant, and with different agents. These aggregations are not infrequently seen in living cells under other natural or experimental depressing conditions and undoubtedly result from the activity of the living protoplasm. The occurrence of death, however, is indicated by the rapid diffusion of the pigment out of the chromatophores and into the vacuole of the cell which becomes a brilliant rose pink by transmitted light and with the loss of the pigment the greenish color of the chlorophyl becomes visible in the protoplasm. By reflected light cells which have undergone this change appear orange yellow and opaque. Diffusion of the pigment to the exterior may be very slow, but there can be no doubt that this change marks the death of the protoplasm, and it is so striking that its beginning and course can be followed without the least difficulty.

In examination of the gradient in *Griffithsia* the substances KCN $m/50$, $m/100$; ethyl alcohol, 10 per cent., 5 per cent.; ethyl ether, 3 per cent., 2 per cent., 1.5 per cent.; HgCl $m/500,000$, $m/250,000$, $m/50,000$, $m/1,000$; $CuSO_4$ $m/50,000$ approx., have been used and some observations on the axial differences in susceptibility to high temperature and confinement have been made.

Within certain limits all these agents and conditions give the same results in axes which are in good physiological condition and in the active vegetative stage. The apical cell is most susceptible, and the course of death is basipetal from cell to cell and usually within the single cell in the more apical regions. In the plants examined most of the older axes consisted of 12–20 cells and the gradient is very often perfectly regular in the first 5–8 cells from the apex downward. Below this modifications and irregularities become more frequent, though the general gradient is often very regular all the way to the base. In the basal half of such axes the cells have commonly undergone a

secondary elongation at the basal end and these cells often show a double gradient, *i. e.*, the apical and basal ends are regions of highest susceptibility and death progresses toward the middle or a region somewhat below the middle of the cell. This appearance of a secondary region of high susceptibility in the basal part of a single cell where secondary growth is occurring is paralleled in multicellular axes where secondary growth occurs in the basal region as in *Ectocarpus* (Child, '16a), and a similar phenomenon appears in many of the lower animals as a secondary growing region at the basal posterior end, which may give rise to new individuals (Child, '13b) or to segments (Hyman, '16). Sometimes the gradient in the elongated cells of the basal region is completely reversed.

The rhizoid of *Griffithsia* possesses a susceptibility gradient, the apical end, the tip of the rhizoid, being the region of highest susceptibility. In general the susceptibility of the apical end of the rhizoid is considerably lower than that of the apical cell of the vegetative axis. In these respects the rhizoid shows much the same physiological relation to other parts as does the "stolon" in the hydroid *Tubularia* (Child, '15c, pp. 91–92, 132–133).

In *Griffithsia*, however, the degree of individuation (Child, '15b, Chap. IX.) is not high, the axial gradient is not very permanently recorded in the protoplasm and therefore readily undergoes modification under altered external conditions. It is possible to eliminate or reverse the gradient experimentally in various ways, *e. g.*, by exposure to high temperature, and plants or cells which have been injured or have been living under unfavorable conditions show alterations of the primary gradient. In general in the vegetative stages the more nearly normal the physiological condition, the more distinctly and uniformly basipetal the gradient. An account of experiments along this line in which one external agent is used to alter the gradient in susceptibility to another is postponed to another time.

In addition to these alterations certain agents in certain concentrations alter the susceptibility gradient to themselves. For example in $HgCl_2$ $m/500,000$ the normal basipetal gradient appears. In $m/50,000$, however, and in higher concentrations there is more or less reversal in the apical region, *i. e.*, the apical cell

and often one, two or three cells next below it are less susceptible than any other part of the axis, and in this group of cells the gradient is usually acropetal, the apical cell being least susceptible of all. Similar results are obtained with $CuSO_4$. This partial reversal is a characteristic feature of susceptibility to concentrations above a certain limit of agents which are powerful coagulants of protoplasm such as $HgCl_2$ and $CuSO_4$. Acclimation is not concerned here, for it is the higher concentrations not the lower which produce the reversal. Apparently these agents decrease the permeability of the cells to themselves and the decrease is greatest in the most apical cells, where the protoplasm is most susceptible to alteration. This differential action of such agents is itself another demonstration of the existence of the gradient, and it is of interest to note that an external agent can reverse the axial gradient in permeability to itself. Various data indicate that other agents in sufficiently high concentration will give similar results, but the details are not yet worked out.

Age differences in the susceptibility of the apical as well as other cells are evident in *Griffithsia*. In general a small apical cell, *i. e.*, the cell which has more recently undergone division, is more susceptible than a larger apical cell, which has passed through a longer period of growth without division. Since different apical cells may be subjected to different external or internal conditions which influence their activity these comparisons often show exceptions to the general rule. The most uniform results as regards these age differences are obtained with a single main axis bearing a number of branches. In such a system the susceptibility of the apical cells usually varies inversely as the size.

A few observations on the form known as var. *tenuis* or as *Griffithsia tenuis*, with greatly elongated slender cells, gave results similar to these already described.

Cystoclonium, Agardhiella, Chondrus, Lomentaria.

In these forms each apparently simple stem or branch represents the orderly growth-activity of one or more monosiphonous axes and their branches, *i. e.*, each macroscopically simple axis is in reality a complex system of monosiphonous axes. In

Cystoclonium and *Agardhiella* each macroscopic axis consists of a single monosiphonous axis with its branches and the vegetative tip is a single cell, while in *Chondrus* and *Lomentaria* each macroscopic axis consists of a number of monosiphonous axes and their branches, and the vegetative tip consists of a group of cells, the apical cells of the main monosiphonous axes. Since these plants show very definite macroscopic axiation, it is of interest to determine whether general axial gradients exist in these axes. Such gradients correspond to the general gradient in the system of main axis and branches of *Callithamnion*. The use of neutral red is not necessary with these four genera, for the changes in color of the phycoerythrin with different killing agents and its diffusion out of the cells indicate very clearly the differences in susceptibility in different regions.

Cystoclonium purpurascens, in the few fronds examined, was found to possess a very uniform basipetal susceptibility gradient, both in the single axes and in the frond in general. Some branches of the frond are very evidently inhibited in their growth and remain short (Kurztriebe) and it is of interest to note that such branches almost always show a lower susceptibility than those which have undergone more rapid growth.

In *Agardhiella tenera* the gradient is also very uniformly basipetal in each axis for several millimeters below the apical end. In KCN $m/50$ which is of course alkaline, the color changes from the normal reddish brown to orange yellow, and this gradually changes to green as the pigment diffuses out. In HCl $m/5$ it first becomes deep purple, and this gradually fades to a dull purplish white with the loss of the pigment. In HCl a distinct basipetal decrease in cell turgor precedes slightly the first change in color, and in KCN accompanies it.

In well developed fronds the purple color begins to appear apically after 15–30 minutes in HCl $m/5$, and after two hours the whole frond has become purple and the apical regions are fading to whitish. In KCN $m/50$ the apical regions begin to turn yellow after about one hour, and after 6–7 hours the change has passed over the whole frond and the extreme apical regions show a slight greenish tint. The final change to purplish white in HCl and to green in KCN is complete only after one or two days.

In regions more than 5–10 mm. from the apical end of the axes the color change is often somewhat irregular and appears first in small areas scattered for some distance along the branch, but even in these regions the progress of death is in general basipetal. At these levels secondary growth in thickness is occurring and it may be that the areas of higher susceptibility represent the apical ends of groups of the monosiphonous axes composing the plant body, which are growing more rapidly than others about them.

In large fronds 15–25 centimeters long the middle regions of the main branches or stems for several centimeters are very commonly less susceptible than either more apical or more basal regions. That the low susceptibility of this region represents a real difference in physiological condition in fronds where it is present is clearly shown by the fact that it is thickly covered with the colorless unicellular hairs characteristic of the species while other parts of the plant show few or none of these hairs. Usually also the color is somewhat lighter than that of other parts of the plant. Undoubtedly this region of low susceptibility is of secondary origin since the younger fronds and main branches do not show it, and the fact that in the plants examined it was limited to these parts of the main branches and stems which were most thickly surrounded by other branches suggests that it may be merely a result of insufficient light or oxygen, or possibly of injurious metabolic products, in other words that it is an incidental result of the crowding of the numerous axes in this region.

The great development of hairs in these regions of low susceptibility suggest that hair development is associated with a low metabolic rate in the cells from which the hairs arise. If this suggestion is correct, the hairs appear first in this middle region because for some reason the metabolic rate is lower there than elsewhere. As the plant becomes physiologically older and its metabolic rate in other regions decreases, hairs may of course appear elsewhere. I have found that plants thickly covered with hairs usually show a lower susceptibility than those with few or no hairs.

In neutral red partial reversals of the gradient in the extreme

apical regions have been observed and in low concentrations of KCN $m/500$ the color-change begins only after 3-5 hours and appears first in the middle region of the main branches and stems, *i. e.*, the regions which are least susceptible to the higher concentrations. From these regions it progresses acropetally and basipetally the tips of the branches being usually the last parts affected. With concentrations of KCN between $m/500$ and $m/50$ or in plants which are somewhat more susceptible to $m/500$, mixed gradients may appear. The color-change may begin and progress basipetally in the apical regions and later it may begin in the middle region and progress more or less acropetally. In plants kept in the laboratory for several days the gradient shows a partial reversal, the susceptibility of the apical 3-5 millimeters of many branches being lower than that of the levels next below.

Chondrus crispus, the common "Iceland moss" with flattened dichotomously branching body, shows a very beautiful basipetal gradient in KCN $m/50$, alcohol 5 per cent., and HCl $m/10$. The first change in color from the deep red-brown or purple-brown appears in the median apical region of each ultimate branch and progresses laterally and basally in extremely regular manner. In KCN this first change is to whitish green, in alcohol to a rose-red or pink, in HCl to a fine violet or purple. Following this change in color there is gradual loss of the pigment by diffusion to the exterior, and the plant becomes whitish and finally almost pure white in KCN and alcohol and white with a trace of purple in HCl. This loss of pigment also begins in the apical region and progresses basipetally. In the concentrations mentioned above the first change in color begins in 1-4 hours and after 30-40 hours the loss of color is complete even to the base.

In plants which are in bad condition, those which have been torn loose and washed about by waves, the susceptibility is in general lower and often lowest of all in the apical regions. Where part of a frond has been torn or broken off and new axes have recently regenerated on the old basal portion the young axes show a much higher susceptibility than the old portion.

Tests of susceptibility with KCN $m/50$ and $HgCl_2$ $m/50,000$ both without and after neutral red, and with neutral red alone, made on a few plants of *Lomentaria uncinata* found detached in

shallow water after a storm showed in most axes the usual basipetal gradient, but in some cases the progress of death was irregular or even acropetal. In most species examined plants detached and washed in by the waves show reversals and irregularities much more frequently than those collected *in situ*, and in *Lomentaria* the irregularities observed are doubtless due to bad condition, but since this species was not found *in situ* there was no opportunity for checking the results.

General Discussion.

From the data recorded here and in the preceding paper it is evident that a gradient in susceptibility is a characteristic feature of the thalli of axiate forms among algæ. In the cases described in the present paper the apical region is primarily the region of highest susceptibility and the decrease is basipetal in each axis. Under unfavorable conditions and in many cases with advancing age, this primary gradient may be altered by local alterations in metabolic activity, by physiological isolation of certain regions, and in many other ways.

In some plant axes the growing tip and the region of highest susceptibility are at the attached or morphologically speaking the basal end and the susceptibility decreases toward the free "apical" end. Some cases of this sort are found in the hairs of certain algæ, *e. g.*, *Fucus* and will be considered at another time.

The significance of the axial differences in susceptibility as indicators of general metabolic rate or condition has been sufficiently discussed elsewhere (Child, '13a, '15b, Chap. III., IX., '15c, Chap. III.). The similarity of results with different agents shows very clearly that the general susceptibility relations depend not upon the specific chemical constitution of a particular agent, but rather upon the fact that many different agents injure and kill protoplasm and that the physiological or metabolic condition, vitality, or whatever term we prefer to use, is a factor in determining their effectiveness as killing agents.

In considering alterations of the gradient it is necessary to distinguish those which occur in low concentrations of KCN and other highly toxic agents or in slightly toxic agents such as neutral red, from those which occur in high concentrations of highly toxic agents such as $HgCl_2$ and $CuSO_4$.

There is first the possibility that the reversals in low concentrations and slightly toxic agents, _e. g._, in _Cladophora_ and _Enteromorpha_ with neutral red and in _Agardhiella_ in neutral red and KCN $m/500$, represent a partial acclimation. In the lower animals the capacity for acclimation varies directly with the metabolic rate or condition along the axis, so that in sufficiently low concentrations the death gradient may be reversed (Child, '13a, '13b, '15b, Chap. III., '16c). True acclimation to a depressing agent or condition consists in a greater or less degree of recovery and approach to the original metabolic condition in the presence of the agent, and in general the capacity for acclimation varies directly with the original metabolic condition. In such cases the reversal of the death gradient does not represent a reversal of the original metabolic gradient along the axis, but is due to the fact that the regions of higher metabolic rate are able to adapt themselves or acquire a tolerance to the agent more rapidly and to a greater degree and so in the long run live longer than regions of lower rate.

Whether these reversals are cases of true acclimation or merely cases in which the primary effect of the toxic agent on the region originally most susceptible alters it in such a way and to such an extent that it becomes less susceptible than other regions to further toxic action must be left for further investigation to determine. It seems probable that at least some agents in certain concentrations too high for acclimation, but not high enough to kill rapidly may actually reverse the susceptibility gradient to themselves possibly through a differential decrease in permeability or an increase in aggregation of the protoplasm or in some other way.

It may be pointed out in this connection that the reversal in _Cladophora_ and _Enteromorpha_ to neutral red can scarcely be the result of reversal of a permeability gradient by the action of neutral red from without for the apical regions apparently take up more neutral red than other parts, but are able to resist its action longer than other parts. In these cases the reversal must result from changes which occur after the neutral red has entered the cells. The reversal to low concentrations of KCN in _Agardhiella_ is also probably not primarily a surface action, for

we should expect such action to be more marked with high rather than with low concentrations.

The more or less complete reversal of the gradient observed in *Griffithsia* with the higher concentrations of $HgCl_2$ and $CuSO_4$ is evidently not identical with the preceding cases of reversal, but is probably due to a decrease in permeability to the killing agent resulting from the action of the high concentrations on the surface of the protoplast. The fact that under such conditions a more or less complete reversal of the susceptibility gradient results means that in the most active protoplasm the permeability is decreased to a very much greater extent than in the less active cells, so that even the agent which has produced the surface change is more completely excluded from those cells where the change is greatest.

These data concerning reversal of the gradient are fragmentary because attention has been directed chiefly to the demonstration of the primary or normal gradient. Further investigation of the changes and the conditions under which they occur will undoubtedly throw more light on the problems involved.

Changes in the axial gradient may also occur in the life of the plant and may be brought about in other ways than those already described. Some of these are merely the result of local action, for example a wound may reverse the gradient, at least temporarily, in regions apical to it. Other changes are due to the action of general external factors as in the case of more or less complete reversal in *Griffithsia* by exposure to high temperature. In this case the high temperature acts like the various toxic agents in high concentration, *i. e.*, the susceptibility gradient to high temperature is basipetal.

Plants which are found detached in shallow water along the shore after storms often show more or less irregularity or reversal of the gradient, undoubtedly in consequence of depressing environmental conditions, such as exposure to high temperature, intense light or drying at low tide. It is quite unsafe to base conclusions on such plants alone. In *Griffithsia* for example, a rather sensitive form, all plants collected along shore after detachment, so far as examined, show more or less reversal in the apical regions, *i. e.*, these regions have been depressed or injured

more than others. Such highly resistant forms as *Ceramium rubrum* (Child, '16*a*), however, usually show the same gradient in detached specimens as in those collected *in situ*. In fact the frequency of irregularities and reversals in the gradient and the ease with which they can be induced experimentally constitute in some degree a measure of the sensitiveness of the species to changes in environment. Experiments on *Griffithsia* to be described later will show some of the possibilities in this direction. All of these cases of alteration or reversal of the gradient, whatever the processes and conditions involved, are of interest in the present connection since they all constitute additional evidence for the existence of a gradient and its fundamental relation to the physiological condition of the plant, and the establishment of these facts is the chief purpose of these studies of algæ.

The visible death changes in the protoplasm of cells stained with neutral red and then killed either with neutral red itself or some other agent consist, as already noted (Child, '16*a*), first, in a deepening of the red color of the dye, indicating increased acidity, followed by an aggregation of the protoplasm into separate masses which rapidly contract and become black or purple. Apparently during this stage of the process there is an increase in acidity in the cell as indicated by the change in color of the neutral red, but this is followed by a more or less rapid loss of color from the masses of coagulated protoplasm and at least often the cell-contents apparently become alkaline, if the neutral red can be trusted as an indicator.

These death changes are most striking in elongated cell bodies and are clearly seen in *Bryopsis*, various species of *Callithamnion*, the hairs of *Chondria*, *Polysiphonia*, *Griffithsia*, etc., but death may occur without such extreme physical changes in the protoplasm, as in the cells of the thallus of *Griffithsia*. It seems probable that the changes characteristic of *Bryopsis*, *Callithamnion* and of the hairs of various forms occur where the layer of protoplasm is very thin and perhaps contains a high percentage of water, while the cells with thicker or a less fluid wall die without exhibiting such extreme physical changes.

The observations on susceptibility gradients in single cells in *Bryopsis*, *Callithamnion* and *Griffithsia* show very clearly that in

a continuous mass of protoplasm very considerable local, or in this case axial, differences in physiological condition and metabolic activity may exist.

In conclusion, the essential similarity of animals and plants in respect to these axial susceptibility gradients may once more be emphasized. The physiological axis is fundamentally the same as regards susceptibility relations in both groups and undergoes very similar alterations.

Summary.

1. The thalli of all axiate algæ examined show an axial gradient in susceptibility to various agents, KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, high temperature, etc. To concentrations or intensities sufficient to kill rapidly without acclimation the apical region is most susceptible, and the susceptibility decreases basipetally in each axis. This susceptibility gradient may undergo more or less complete reversal under various conditions. Certain concentrations of certain agents may even reverse the gradient in susceptibility to themselves.

2. As in animals the susceptibility gradient is in general an indicator of the vitality, metabolic rate, or physiological condition at different levels of the axis. The gradient may be altered or more or less completely reversed by change in external conditions, by advancing age, by physiological isolation of parts, etc., and the readiness with which alterations occur in altered environment is in some degree a measure of the sensitiveness of the species.

Hull Zoölogical Laboratory,
    University of Chicago,
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